

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k033861

B. Analyte:

Legionella pneumophila DNA

C. Type of Test:

DNA amplification test

D. Applicant:

Becton, Dickinson and Company

E. Proprietary and Established Names:

BD ProbeTec™ ET *Legionella pneumophila* (LP) Amplified DNA Assay

F. Regulatory Information:

1. Regulation section:
21 CFR 866.3300; Haemophilus spp. Serological Reagents
2. Classification:
Class II
3. Product Code:
LQH; DNA-reagents, Legionella
4. Panel:
Microbiology (83)

G. Intended Use:

1. Intended use(s):
The BD ProbeTec™ ET *Legionella pneumophila* (LP) Amplified DNA Assay, for use with the BD ProbeTec ET System, employs Strand Displacement Amplification (SDA) technology for the direct qualitative detection of *Legionella pneumophila* DNA (serogroups 1-14) in sputum specimens from patients with a clinical suspicion of pneumonia. It is intended to aid in the presumptive diagnosis of Legionnaires' disease in conjunction with culture and other methods.
2. Indication(s) for use:
The BD ProbeTec™ ET *Legionella pneumophila* (LP) Amplified DNA Assay, for use with the BD ProbeTec ET System, employs Strand Displacement Amplification (SDA) technology for the direct qualitative detection of *Legionella pneumophila* DNA (serogroups 1-14) in sputum specimens from patients with a clinical suspicion of pneumonia. It is intended

to aid in the presumptive diagnosis of Legionnaires' disease in conjunction with culture and other methods.

3. Special condition for use statement(s):
Not applicable
4. Special instrument Requirements:
BD ProbeTec™ System

H. Device Description:

The Assay is based on the simultaneous amplification and detection of target DNA sequences using nucleic acid primers and fluorescently-labeled detector probes in a process known as strand displacement amplification (SDA). The SDA reagents are dried in two separate disposable microwells. Processed sample containing DNA is added to a Priming Microwell, which contains the amplification primers, fluorescently-labeled detector probes, and other reagents necessary for amplification. The primers amplify an 84 base pair region of the *L. pneumophila* macrophage infectivity potentiator (mip) gene that is conserved across all serogroups of the species. Following incubation, the reaction mixture is transferred to an Amplification Microwell, which contains two enzymes (a DNA polymerase and a restriction endonuclease) necessary for SDA. The Amplification Microwells are sealed to prevent contamination and then incubated in a thermally controlled fluorescent reader, which monitors each reaction for the generation of amplified products. Each reaction co-amplifies and detects an Internal Amplification Control (IAC), as well as the target DNA. The purpose of the IAC is to verify that proper conditions exist for amplification and to reduce the possibility of reporting a false negative result due to specimen inhibitors. The presence or absence of *L. pneumophila* DNA is determined by calculating PAT scores (Passes After Threshold) for the specimen based on predefined threshold values. The instrument automatically reports the results as positive, negative or indeterminate.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Binax NOW™ Legionella Urinary Test
2. Predicate K number(s):
k934965
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Procedure	Qualitative; detection of <i>Legionella pneumophila</i>	Qualitative; detection of <i>Legionella pneumophila</i>
Differences		
Item	Device	Predicate
Assay	Nucleic acid amplification and probe detection	Enzyme Immunoassay (EIA), membrane assay
Specimen Type	Sputum	Urine

J. Standard/Guidance Document Referenced (if applicable):

Not applicable

K. Test Principle:

Strand displacement amplification (SDA)

L. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

Reproducibility of the BD ProbeTec™ ET LP Assay was assessed at three laboratories by testing a panel consisting of 24 samples seeded in PBS/BSA. The panel comprised 12 samples that were negative for *L. pneumophila*; three low (300 CFU/reaction) and three high (500 CFU/reaction) positive samples of *L. pneumophila*; and three low (30 Elementary Bodies (EB)/reaction, 300 CFU/reaction, 900 cells/reaction, respectively) and three high (50 EB/reaction, 500 CFU/reaction, 1500 cells/ reaction, respectively) positive samples each containing *Chlamydomphila pneumoniae* (CP), *L. pneumophila*, and *Mycoplasma pneumoniae* (MP). The %CV varied from 2 to 6%.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability (controls, calibrators, or method):*

Not applicable

d. *Detection limit:*

The analytical sensitivity of the BD ProbeTec™ ET LP Assay across 14 different serogroups of *L. pneumophila* was determined by diluting bacterial suspensions to levels of 0, 150, 300 and 450 Colony Forming Units (CFU) per reaction. Based on 100% positivity, the analytical sensitivity for serogroups 2-10 and 12 was 150 CFU/reaction. The analytical sensitivity for serogroups 1 and 11

was 300 CFU/reaction; serogroup 13 was 450 CFU/reaction; and serogroup 14 was 700 CFU/reaction.

The analytical sensitivity of the BD ProbeTec™ ET LP Assay in the presence of a lower respiratory specimen matrix was determined by seeding a macrophage-infected stock of *L. pneumophila* serogroup 1 into sputum at levels of 0, 150, 300 and 450 CFU per reaction. The specimens were processed according to the lower respiratory processing procedure and assayed in triplicate. Based on 100% positivity, the analytical sensitivity for lower respiratory specimens was 150 CFU/reaction.

e. Analytical specificity:

A total of 79 microorganisms (69 bacteria, one yeast and nine viruses) were evaluated using the BD ProbeTec™ ET LP Assay. Bacterial isolates were tested at concentrations ranging from 1×10^6 CFU/mL to 1×10^8 CFU/mL. Viruses were tested at a concentration of 1×10^6 viral particles/mL. None of the microorganisms tested produced a positive result or inhibited the Internal Amplification Control (IAC).

f. Assay cut-off:

The threshold values for both the *L. pneumophila* target DNA and the IAC were initially established using Receiver Operator Characteristic curve analyses of data obtained from positive and negative controls. These threshold values were verified in clinical studies and with retrospective *L. pneumophila* positive and negative specimens.

2. Comparison studies:

a. Method comparison with predicate device:

Study 1: The BD ProbeTec™ ET LP Assay was evaluated prospectively at seven clinical centers within the United States and Canada during the 2002 –2003 respiratory season. A sputum specimen and a urine specimen were collected from each patient. Sputum specimens were tested using the BD ProbeTec™ ET LP Assay, *Legionella* culture, and a Direct Fluorescent Antibody assay (DFA). Urine specimens were tested using a commercially available *L. pneumophila* urinary antigen assay.

A total of 114 sputum specimens collected from 114 patients met the criteria for inclusion in the study (i.e., radiographic evidence of pneumonia, patients ≥ 13 years of age, on antibiotics ≤ 14 days, valid specimen types collected, appropriate reference methods conducted, etc.). Results of the sputum specimens were compared to the culture result to estimate performance characteristics. There were no positive specimens found by culture or Probe Tec™ ET LP Assay. The specificity was 100% (114/114).

Study 2: The BD ProbeTec™ ET LP Assay was also evaluated with 83 retrospective sputum specimens acquired from clinical sites within the United States, Europe, and Canada. Each site tested the sputum specimens in the BD ProbeTec™ ET LP Assay and compared the results to the culture result. The positive agreement was 91.3% (21/23), the negative agreement was 86.7% (52/60) and the overall agreement was 88 % (73/83).

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a and b are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Not applicable

M. Conclusion:

The Performance characteristics reported here for the device indicates that it is comparable to other such test kits currently in the market.